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PRINCIPAL INVESTIGATOR: Gertraud Maskarinec, Ph.D.

CONTRACTING ORGANIZATION: University of Hawaii
Honolulu, Hawaii 96822

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#### **FOREWORD**

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#### Introduction

A possible association between mammographic parenchymal patterns and breast cancer risk has been shown in numerous studies<sup>1-4</sup>. Parenchymal patterns refer to the distribution of fat and connective and epithelial tissue in the female breast. Fat appears dark on mammograms and the other tissues appear light. Since fat tissue is not at risk to become cancerous, the radiographically light areas, the mammographic densities, are thought to be relevant to breast cancer risk. Monitoring changes in mammographic density might offer a non-invasive method to evaluate preventive strategies and to select populations at high cancer risk for interventions. A recent publication from Canada<sup>5</sup> reported a decrease in mammographic density patterns as a result of a low-fat, high-carbohydrate dietary intervention.

Based on the observation that breast cancer incidence rates differ among ethnic groups in Hawaii, our study hypothesis is that mammographic density patterns vary among women from different ethnic groups. In 1986-1990, the breast cancer incidence rate (age-adjusted to 1970 U.S. population) for Caucasians was 133/10<sup>5</sup>, for Hawaiian/Part Hawaiians 113/10<sup>5</sup>, for Japanese 89/10<sup>5</sup>, for Filipina 57/10<sup>5</sup>, and for Chinese 58/10<sup>5</sup> (Hawaii Tumor Registry, unpublished report). Therefore, it was proposed that women from ethnic groups with high breast cancer risk are more likely to have a dense parenchymal pattern than women from ethnic groups at low risk for breast cancer. Intake of soy products was suggested as one of the dietary factors that may protect Asian women from breast cancer<sup>6</sup>.

The purpose of this study is to determine whether mammographic density patterns differ by ethnic background and to explore the possible association of a soy rich diet with mammographic density patterns. Asian populations have traditionally consumed large amounts of soybeans, a major source of phytoestrogens, in particular isoflavones, heterocyclic phenols similar in structure to natural and synthetic estrogens<sup>6,7</sup>. The hypothesis that these weakly estrogenic substances may exhibit anti-estrogenic properties by competing for estrogen receptors and protect women against breast cancer has been supported by animal studies<sup>7,8</sup> demonstrating anti-estrogenic and antineoplastic effects. Recently, a first report of a population based study<sup>9</sup> assessing exposure to phytoestrogens through urinary analysis was published.

The specific objectives of this project are to determine whether mammographic density patterns differ among women of Japanese, Filipino, Hawaiian, and Caucasian ancestry after adjusting for age, family history of breast cancer, parity, menopausal status, hormone replacement therapy, weight, and height, to explore the possible association of a soy rich diet on mammographic parenchymal patterns while controlling for confounding variables, and to investigate the association between urinary isoflavone levels and self-reported soy intake.

#### Methods

Recruitment: Women have been recruited at three mammography facilities in four locations on the Island of Oahu: Kapiolani Women's Center, Kaiser Permanente Honolulu and Punawai Clinics, and St. Francis Hospital. We recently included St. Francis Hospital because of the large number of Filipino patients frequenting this institution. The recruitment procedures differ among

clinics. Kapiolani Women's Center mails a flyer (see appendix) with the letter confirming the mammography appointment. The short text explains the purpose of the study and the involvement expected from potential participants. If a woman is interested in the study, she will return the flyer to the mammography technician at the time of the appointment. Using the last name, first name, and date of birth, we evaluate the corresponding record in the Health Information Query database of the Kapiolani Women's Center. We only recruit women with a healthy mammogram from this clinic. Mammography technicians at the Kaiser clinics are helping with the recruitment by asking women who have a mammogram appointment to read the flyer, while waiting for their mammogram. If a woman is interested in the study, she will leave the paper with contact information in the collection box. In addition, we do in person recruitment at the Honolulu clinic. Every week, one of the research assistants spends a few hours in the waiting room and explains the study to women who wait for their appointment. If a woman agrees to participate, she is given the agreement, the soy questionnaire, the diet questionnaire, and a stamped returning envelope. In St. Francis Hospital, recruitment is performed with the help of mammography technicians.

Every week, we collect the flyers from all mammography clinics. After the contact information is added to our recruitment database, the questionnaires and informed consent form are mailed to each prospective participant. After a woman finishes the self-administrated questionnaires and signs the agreement to participate, she returns these documents by mail. Women who do not return the questionnaire within two weeks are reminded by telephone up to 3 times. Data from the soy questionnaires is entered into a computer manually, whereas the food frequency questionnaires are scanned electronically. A dietary analysis (see appendix) is printed for every participating woman. We mail a copy of the consent form, the dietary analysis, and a copy of dietary guidelines on eating right to each participant.

Nutritional analysis: All study participants completed a soy food questionnaire (see appendix) asking for consumption of soy products during the last year. All women who donated a urine sample were also asked to complete an additional soy intake questionnaire reporting their soy intake during the last 24 hours before urine collection. Using nutritional information from the U.S. Department of Agriculture's Food Composition Database, Release 10<sup>10</sup>, we estimate the daily intake of soy protein for each participant. First, information on the frequency of consumption, the average serving size, and the protein content is multiplied for each food item. Then the estimated daily soy intake is added across all food items. Total daily isoflavone intake is estimated for each subject using the same method. The information on isoflavone concentrations in the different food items was provided by Dr. Adrian Franke at the Cancer Research Center of Hawaii<sup>11</sup>, who has performed repeated analyses of soy products available in Hawaii.

Urine collection: An overnight urine sample was collected for 102 study participants. All 132 women who had been recruited in July were contacted for a urine sample. Of these, 5 women refused to provide a sample, 2 women were unable due to menstruation, 6 women lived too far away, 8 women were on vacation, and 9 women could not be reached by telephone. A container with .1 - .3 g of ascorbic acid and .2 - .4 g of boric acid (to prevent bacterial contamination and degradation of analytes) was delivered to each women at their home or work place by a research

assistant. Women were instructed to collect to first urine sample in the morning and all samples if they urinated during the night after going to bed. They were asked to record the time of last urination and the time of the actual urine collection. The containers were collected in the morning and transported in an ice box to the laboratory. After mixing and weighing of each urine sample, the urine was transferred into three 25 ml disposable plastic tubes. The aliquot samples were stored between -20°c and 70°c until analyzed.

*Urine analysis:* Urine samples were analyzed at Dr. Franke' lab at the Cancer Research Center of Hawaii. Analysis was done by diode-array reversed-phase HPLC (High Performance Liquid Chromatography) method for the most common dietary isoflavones daidzein, genistein, glycitein and their metabolites equol, coumestrol and O-desmethylangolensin (DMA). Frozen urine samples were thawed and analyzed in batches according to the published method<sup>12</sup>.

Mammogram density assessment: Mammograms were requested in batches from the clinics after the radiologists had completed their evaluation. The two cranio-caudal mammogram films were scanned into a PC, computerized mammographic density assessment was performed<sup>13</sup>. The reader first draws the outline of the breast (using an outlining tool) and then searches for the best threshold gray level value X where all pixels with values above X are considered to represent mammographic densities. The pixel count corresponding to the area colored within the outline of the breast is determined by the computer, as is the total area within the outline of the breast. The proportion of the breast with densities is calculated as the ratio of the colored area to the total area of the breast. The measurements for three readers were averaged. Based on the first 39 mammograms, correlation coefficients between three readings were greater than 0.9.

During the last year, we have met three times with Dr. Giske Ursin from the University of Southern California, who trained us in the computerized assessment method developed at her institution. In addition, we observed Dr. Norman Boyd's method in Toronto. Scanning of mammograms was temporarily discontinued when we noticed that the computer images of some of the darker mammographic films did not show a very discrete skin line. The skin line is needed to measure the size of the entire breast. In consultation with the manufacturer of the X-ray digitizer, we learnt to adjust the settings of the software to improve image quality. However, to obtain quality images for even the darkest films, it was necessary to replace the Cobrascan CX 312-T with an upgraded model specifically designed for mammograms. The new scanner should be installed by the beginning of October.

Statistical analysis: Because dietary soy intake and urinary isoflavone levels were skewed, we used a logarithmic transformation to normalize the distribution. To increase the size of subgroups for analysis, women with Caucasian and native Hawaiian ancestry were combined as one group and Japanese, Chinese, and other Asian women as another group. Student's t-tests and  $\chi^2$  tests<sup>14</sup> were applied to assess differences between ethnic groups. Pearson's correlation coefficient and multiple linear regression models were used to explore relations between variables. All analyses were performed using PC-SAS®, release 6.12 (SAS Institute, Cary, NC).

### **Preliminary Results**

Recruitment: By September 22, 1997, 486 women had indicated their interest in our study by completing one of the flyers. Of these women, 243 were recruited at Kapiolani Women's Center, 223 at the Kaiser Honolulu Clinic, 13 at the Kaiser Punawai Clinic, and 7 at St. Francis Hospital. By the same date, 253 women, 150 at Kapiolani Women's Center, 97 at the Kaiser Honolulu Clinic, 6 at the Kaiser Punawai Clinic, and none at St. Francis Hospital) had returned the completed questionnaires and consent forms. Based on our return rate so far, we estimate the response rate to lie between 60% and 70%.

The median age of the 253 women who returned the questionnaires was 50 years (range: 34 to 84 years). The ethnic distribution was as follows: 102 (40.3%) Caucasian, 32 (12.6%) Chinese, 9 (3.6%) Filipino, 28 (11.1%) Native Hawaiian, 77 (30.4%) Japanese, and 5 (2%) others. The mean soy protein intake was estimated at 3.7 g per day with a maximum of 33 g per day. Approximately half of the participants reported a soy intake of less than 2 g per day and 25% of women more than 4.5 g per day. The ethnic groups were combined into two groups, one for women at high breast cancer risk (Caucasian, Native Hawaiian) and at low breast cancer risk (all Asian). Dietary soy and isoflavone intake differed significantly between the two groups (Table 1). The other dietary data has not been analyzed yet.

Table 1. Age and Dietary Information for 253 Women

Ethnicity	Caucasian/Hawaiian N=130		Asi N=	p	
	Mean	SD	Mean	SD	
Age (years)	52.7	10.4	52.3	10.9	< 0.05
Soy protein (g/day)	2.6	4.1	4.8	5.4	0.0004
Dietary isoflavones (mg/day)	7.4	13.8	14.3	19.4	< 0.0001

*Urine analysis:* We collected urine samples from 102 women, but had to exclude one woman from the analysis because she did not complete the soy questionnaire. The mean isoflavone level was 323 nmol/hour (range: 0 - 3995 nmol/hour). As in the larger sample, soy intake was significantly higher among women with Asian ancestry than among women with Caucasian and Hawaiian ancestry (Table 3). Self-reported soy protein intake during the last year and during the last 24 hours showed a strong correlation (r=0.49, p=0.0001), but was higher during the last 24 hours. The two groups were similar in age structure. Soy intake and urinary isoflavone levels were more than twice as high in Asian women as in Caucasian and Hawaiian women.

The correlation coefficient between urinary isoflavone levels and dietary soy intake during the last 24 hours was 0.58 (p=0.0001). The correlation between urinary isoflavone levels and estimated isoflavone intake was very similar with 0.59 (p=0.0001). For dietary soy and

isoflavone intake during the last year, the correlation with urinary isoflavone levels were 0.26 (p=0.01) and 0.27 (0.006) respectively. In a linear regression model with urinary isoflavone levels as dependent variable, ethnicity and soy intake during the last 24 hours explained approximately 38% of the variance.

Table 2. Results of Urinary Isoflavone Analysis and Soy Intake

Ethnicity	Caucasian/ Hawaiian (N=53)		Asi (N=	p	
	Mean	SD	Mean	SD	
Age (years)	52.7	10.4	52.3	10.9	< 0.05
Soy protein (g/day) - last year	2.2	2.9	4.5	4.3	0.003
Soy protein (g/day) - 24 hours	3.7	8.9	8.9	14.7	0.03
Dietary isoflavones (mg/day) - last year	6.6	9.8	15.1	21.0	0.01
Dietary isoflavones (mg/day) - 24 hours	10.1	24.5	28.6	44.2	0.01
Urinary isoflavones (nmol/hour)	182.3	429.5	479.4	774.5	0.02

Mammographic densities: So far, mammographic density analysis has been completed for 39 women. Among this group of women, Asian women were five years older (p<0.05), were more likely to be on estrogen replacement therapy (45% vs. 21%), had a lower body mass index and a three times higher daily consumption of soy protein than Caucasian/Hawaiian women (Table 3).

Table 3. Results of Mammographic Density Assessment by Ethnicity

Ethnicity	Caucasian/Hawaiian (N=19)		Asi (N=	p	
	Mean	SD	Mean	SD	
Area of Breast (pixels)	201,105	101,798	102,576	61,348	< 0.05
Dense Area (pixels)	49,286	27,910	36,519	21,580	0.12
Percent Densities (%)	32%	22%	39%	15%	0.22
Body Mass Index	28.0	6.8	23.4	5.0	0.02
Soy Protein Intake (g/day)	2.3	2.6	7.4	8.6	0.02

No significant differences in parity, age at first live birth, years of breast feeding, age at menarche and at menopause, educational achievement, daily calories, alcohol intake, and calories

from fat were observed between the two groups. The mean area of the breast was nearly twice as large for Caucasian/Hawaiian as for Asian women (Table 3). The mean dense area was also smaller in Asian women, but the difference did not reach statistical significance. In comparison to Caucasian/Hawaiian women, the percentage of densities was slightly higher in Asian women. Excluding women on estrogen replacement therapy did not change the pattern of the results.

#### Discussion

Overall recruitment for this study is proceeding quite well. Within one or two months, we will reach our goal of 400 study participants. However, the number of women recruited from each ethnic group are unequal. Caucasian and Japanese women are the largest groups at this time. The plan was to recruit approximately equal numbers of women from different ethnic groups. One reason for recruiting fewer Chinese, Filipino, and Native Hawaiian women in the clinics, is that population sizes are smaller than for Caucasian and Japanese women. However, the problem is compounded by the fact that Filipino and Native Hawaiian women are less likely to obtain mammograms and more likely to attend clinics outside Honolulu. We tried to add a hospital with many Filipino patients, but recruitment there has been very slow. Language problems are an additional problem for Filipino women. We are thinking of including another mammography clinic outside Honolulu to increase the number of Filipino and Native Hawaiian women in the study.

Urinary isoflavone measurements have been used to validate the nutritional information from the soy questionnaire. We found a fairly high correlation between urinary isoflavone measurements and dietary soy protein and isoflavone intake as estimated from the questionnaire. Therefore, we feel confident that the soy questionnaire is a useful tool to assess regular intake of soy foods.

The preliminary data on mammographic density suggest that the area of dense tissue in the breast may be smaller in Asian than in Caucasian women. However, because of their relatively smaller breast size, the percent of the breast occupied by dense tissue in Asian women may be equal to or higher than in Caucasian women. We need to perform multivariate analysis in a larger population to investigate the relation between ethnicity, diet, known breast cancer risk factors, and mammographic density patterns.

#### **Conclusions**

Preliminary analysis of data collected so far appear to be in support of our hypothesis that women at low risk for breast cancer have fewer mammographic densities. Recruitment for this study is proceeding well and will be completed shortly. Two of us have been trained in mammographic density assessment and will be able to read all mammograms as soon as the upgraded scanner is installed. The collection and analysis of urine samples has been completed. Statistical analysis on available data is under way.

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# Are you concerned about breast cancer? Would you like to contribute to new research? Are you interested to know more about what you are eating?



The Kaiser mammography clinic is assisting the Cancer Research Center of the University of Hawaii to find new ways of preventing breast cancer. Therefore, we encourage you to enroll in a study about diet and the appearance of mammograms. The goal of the study is to learn more about the effect of our diets on normal mammograms.

When you come for your mammography appointment, we will ask you if you are willing to participate in this new study for healthy women. If you are, leave the lower portion of this letter with the mammography technologist and a researcher will contact you during the following four weeks. Participation involves no pain, just a little bit of your time. You will be asked to complete a dietary questionnaire at your own leisure. In addition, the researchers want to analyze your mammogram on a computer after the radiologist at the clinic has found your mammogram to be normal. As an appreciation for your cooperation, you will be provided with a summary of your diet listing the average daily intake of some important nutrients.

We hope you give this study a chance. We need to know so much more in order to prevent breast cancer. And we need your help to protect women from this disease. If you want more information, please call Dr. Gertraud Maskarinec or Ms. Lisa Meng at the University of Hawaii, Cancer Research Center, telephone 586-3078.

Cut here	Cut here
Yes, I am interested. Please contact me	e No, I am not interested.
Date of Mammography:	
Name:	Birth Date:
Address:	
Best Phone Number to call:	Best time to call:
Comments:	

# YOUR AVERAGE DAILY DIETARY INTAKE

FOOD COMPONENTS	YOUR DIET	RECOMMENDED
Calories	1,523 calories	*
Protein	55 grams	50 grams
Calories from fat	24%	Less than 30%
Calories from saturated fat	7%	Less than 10%
Cholesterol	163 mg	Less than 300 mg
Dietary fiber	17 grams	25 grams
Vitamin A	10,424 I.U.	4,000 I.U.
Vitamin E	8 I.U.	12 I.U.
Vitamin C	117 mg	60 mg
Thiamin	1.0 mg	1.0 mg
Riboflavin	1.0 mg	1.2 mg
Niacin	13 mg	13 mg
Vitamin B <sub>6</sub>	2.0 mg	1.6 mg
Folate	212 mcg	180 mcg
Vitamin B <sub>12</sub>	3.0 mcg	2.0 mcg
Calcium	702 mg	800 mg
Phosphorus	1,017 mg	800 mg
Sodium	2,300 mg	Less than 2400 mg
Magnesium	276 mg	280 mg
Iron	9 mg	10 mg
Zinc	6 mg	12 mg
Selenium	76 mcg	55 mcg

<sup>\*</sup> Your calorie intake depends on your desirable weight. Use the pamphlet to check your desirable weight. If your weight is desirable, then you are balancing your calorie intake with your physical activity.

# Questionnaire: Diet Related to Soy Beans

Date:	Date of Birth:	ID:
Last Name:	First Name:	

Please answer the following questions your eating habits during the last 12 months.

For each food group, mark how often you ate those items and then your usual serving size. If you ate some soy foods not listed here, please put them in the "Other" category in the last row.

Thank you very much! We appreciate your help in this study.

		Average Use During Last Year							
Food Item	Never or hardly ever	Once a month	2 to 3 times a month	Once a week	2 to 3 times a week	4 to 6 times a week	Once a day	2 or more times a day	Your Usual Serving Size
Tofu (soybean curd, including plain, in salad, in soups, in mixed dishes)	0	0	0	0	0	0	0	0	O 2 cubes or 1/4 cup O 1/4 block or 1/4 cup O 1/4 block or more
Miso (including in soup, in dressing, in marinated fish or meat)	0	0	0	0	0	0	0	0	O ½ cup O I cup O 2 cups
Green soybeans (including as snack, in mixed dishes)	0	0	0	0	0	0	0	0	O 1/4 cup or I handful O ½ cup O I cup
Fried tofu, such as tau foo pok, yaki-dofu, aburage(including plain, staffed, cone sushi)	0	0	0	0	0	0	0	0	O I piece or 1/4 cup O 2 pieces or 1/4 cup O 1 cup
Tau foo kwa or tofu gan (pressed tofu)	0	0	0	0	0	0	0	0	O 1/2 cup O 1-1/2 cup
Soybean sprouts (including use in cold dishes and hot dishes)	0	0	0	0	0	0	0	0	O 1/4 cup O ½ cup O 1 cup
Foojook or tofu skin	0	0	0	0	0	0	0	0	O 1/4 cup or 1 sheet

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Green soybeans (including as snack, in mixed dishes)	0	0	0	0	0	0	0	0	O 1/4 cup or I handful O % cup O I cup
Fried tofu, such as tau foo pok, yaki-dofu, aburage(including plain, staffed, cone sushi)	0	0	0	0	0	0	0	0	O I piece or 1/4 cup O 2 pieces or 1/4 cup O 1 cup
Tau foo kwa or tofu gan (pressed tofu)	0	0	0	0	0	0	0	0	O ½ cup O I cup O I-1/2 cup
Soybean sprouts (including use in cold dishes and hot dishes)	0	0	0	0	0	0	0	0	O 1/4 cup O ½ cup O 1 cup
Foojook or tofu skin (including in jal, jook, dim sums, mixed dishes, or vegetarian meats)	0	0	0	0	0	0	0	0	O 1/4 cup or 1 sheet O 1/2 cup O 1 cup foojook
Western vegetarian meats made with soy products (such as tofu hot dog, tofutti, soy cheese, etc.)	0	0	0	0	0	0	0	0	O 2 meatballs or ½ cup O I patty or 2/3 cup O I large patty or I cup
Soybean drink or milk (sweet, unsweetened, flavored, salty)	0	0	0	0	0	0	0	0	O ½ cup O 1 cup O 2 cups
Other soy products such as natto (fermented soybeans), roasted soybeans, kuromame(black soybeans), okara, kinako (roasted soybean flour)	0	0	0	0	0	О	0	0	O 1/4 cup O ½ cup O I cup
Other beans than soybeans (such as chili, refried beans, slip pea, azuki beans, lama bean, chick peas, black-eyed peas, pinto beans, broad beans, navy beans, mung beans, etc.)	0	0	0	0	0	0	0	O	O 1/4 cup O ½ cup O 1 cup
Others	О	0	0	0	0	0	0	0	O 1/4 cup O 1/4 cup O 1 cup